Golden hamster (*Mesocricetus auratus*) as an experimental model for *Leishmania* (*Viannia*) *braziliensis* infection

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SUMMARY

The lack of an adequate model for *Leishmania* (*Viannia*) *braziliensis* infection is a limiting factor for studying American tegumentary leishmaniasis (ATL). The golden hamster (*Mesocricetus auratus*) is a promising model because besides being highly susceptible to dermotropic *Leishmania* infection, the lesions are very similar to cutaneous leishmaniasis (CL) in humans. However, different *Leishmania* isolates or species and/or protocols have resulted in different outcomes, whereas no study has evaluated the reproducibility of *L. braziliensis* infection in this model. The natural history of *L. braziliensis* infection in 34 hamsters was evaluated by using a single parasite isolate in 8 independent experiments under similar experimental conditions. Clinical, histological and immunological analyses were performed. The hamsters presented skin ulcers similar to those observed in ATL. The intra-experiment lesion increment tended to show an intermediary variance. Histological analysis of infected skins showed granulomatous reaction, scarce amastigotes, and Schaumann's bodies. Blood lymphocytes proliferated in response to leishmanial antigens. The severity of the infection was positively correlated to spleen weight, and the titres of anti-*Leishmania* IgG antibodies. Our findings indicate that the hamster is an appropriate model for immunopathogenesis studies of CL caused by *L. braziliensis*, supporting its use in clinical, vaccine and chemotherapy experimental protocols.

Key words: golden hamster, American tegumentary leishmaniasis, *Leishmania* (*Viannia*) *braziliensis*, immunoglobulin, lymphocyte, clinical outcome, histopathology.

INTRODUCTION

Leishmania (*Viannia*) *braziliensis* is the most prevalent species associated with American tegumentary leishmaniasis (ATL). ATL is a public health problem with approximately 25 000 cases reported annually in Brazil (SVS-MS, 2011). Nevertheless, there is no vaccine for ATL and only a limited number of drugs are available for treating patients. Most of our knowledge on the immunopathogenesis of *L. braziliensis* infection comes from studies in patients and in asymptomatic individuals (Reithinger *et al*. 2007; Carvalho *et al*. 2012). The lack of an adequate experimental model for *L. braziliensis* is a limiting factor for the development of biological and pharmacological health inputs to ATL.

Although relevant for cutaneous leishmaniasis (CL) studies, murine models (Balb/c and C57Bl/6) are naturally resistant to *L. braziliensis* (DeKrey *et al*. 1998; Rocha *et al*. 2007). When infected by *L. braziliensis,* animals develop small non-ulcerated lesions that show a progression to spontaneous healing (DeKrey *et al*. 1998; Rocha *et al*. 2007). There are quite a few reports employing a Balb/c model in vaccination studies that successfully obtained chronic ulcerated lesions after infection by *L. braziliensis* promastigotes (Salay *et al*. 2007). Other experimental models such as non-human primates (Souza-Lemos *et al*. 2008) and dogs (Pirmez *et al*. 1988) require complex logistics for their maintenance under experimental conditions.

The hamster is highly susceptible to dermotropic *Leishmania* infection and has been largely used as a model for visceral leishmaniasis (Goto and Lindoso, 2004; Dea-Ayuela *et al*. 2007). The animal develops skin lesions when infected by one of the *Viannia* or *Leishmania* species including *L.* (*V.*) *braziliensis* (Brazil, 1976; Wilson *et al*. 1979; Morais-Teixeira *et al*. 2008), *L.* (*L.*) *amazonensis* (Figueiredo *et al*. 1999), *L.* (*V.*) *guyanensis*, L. (*V.*) *panamensis* (Rey *et al*. 1990; Osorio *et al*. 2003), *L.* (*V.*) *lainsoni* (Corrêa *et al*. 2007) and *L.* (*V.*) *peruviana* (Gamboa *et al*. 2008). Indeed, the skin lesions developed by these animals are very similar to the CL ulcers observed in humans (Hommel *et al*. 1995). This turns the golden hamster into a promising model for the study of ATL.

A number of experimental protocols using *Leishmania*-infected hamsters have been described. These protocols differ on several parameters, including the *Leishmania* strains (Wilson *et al*. 1979;

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Rey *et al*. 1990; Kahl *et al*. 1991), number of inoculated parasites (Wilson *et al*. 1979; Martinez *et al*. 1991), route and site of inoculation (Wilson *et al*. 1979; Osorio *et al*. 2003), animal gender or age (Wilson *et al*. 1979; Travi *et al*. 2002) and biological characteristics of inoculated parasites such as the number of *in vitro* passages, growth phase, developmental stages or rate of metacyclic forms (Rey *et al*. 1990; Gamboa *et al*. 2008). Most of the studies using dermotropic strains involved *L. guyanensis*, but the outcome of *L. braziliensis* infection was poorly studied. To date, no study has evaluated the reproducibility of the clinical aspects of the infection in the hamster model for CL caused by *L. braziliensis*.

Here we studied the natural history of *L. braziliensis* infection in a hamster model by using a single parasite isolate in independent experiments under similar experimental conditions. Our findings indicate that the hamster is an appropriate model for *L. braziliensis* infection studies.

MATERIALS AND METHODS

Animals and ethics statement

Outbred golden hamsters (*Mesocricetus auratus*), adult females (6–8 weeks old), weighing 80–90 g, from the animal facilities at Fundação Oswaldo Cruz, were used. Thirty-four infected animals and 13 uninfected animals were analysed. This study was specifically approved by the Ethics Committee on Animal Use (CEUA) of Fundação Oswaldo Cruz – FIOCRUZ, by the number of protocol P-0281/06.

Parasites for infection and immunological studies

Leishmania braziliensis promastigotes (MCAN/BR/98/R619) in stationary growth phase until the third *in vitro* passages in supplemented Schneider's *Drosophila* medium were used (Sigma Chemical Co., St Louis, MO, USA). Promastigotes were washed in phosphate-buffered saline, 0·15 M, pH 7·2 (PBS) and 1×10^6 parasites were inoculated intradermally in the dorsal hind paw of hamsters. Disrupted antigens of *L. braziliensis* (MHOM/BR/75/2903) promastigotes (Lb-Ag) were obtained for immunological studies.

Clinical course of Leishmania braziliensis *infection*

To determine the natural history of *L. braziliensis* infection in hamsters, 8 independent experiments were performed during a period of 2 years. The lesion increment was monitored weekly from day 7 up to approximately 110 days post-infection. This was done by measuring the paw dorsum-ventral thickness with a digital thickness gauge (Mitutoyo America Corporation, São Paulo, Brazil) with the thickness expressed in millimetres. The lesion increment was determined as the difference of measurements between the infected and the non-infected paw of the same animal. The discrepancies in lesion increment were determined by the variance coefficient [VC = (standard deviation × mean)/100)]. For this VC analysis, 5 experiments with 5 or 6 animals per group were used. Once a week animals were checked for skin macroscopic changes and for cutaneous metastasis.

*Quantification of anti-*Leishmania *antibodies*

The anti-*Leishmania* IgG levels were determined in plasma samples by ELISA assay as described elsewhere (Gomes-Silva *et al*. 2008). Plasma samples were diluted 1:200 and horseradish peroxidase-labelled goat anti-hamster IgG was used as detector system (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The results were expressed as optical density (OD).

Lymphocyte proliferative responses to Leishmania *antigens*

Peripheral blood mononuclear cells were collected for lymphocyte proliferative response analysis in response to *Leishmania* antigens as described elsewhere (Da-Cruz *et al*. 2002). The cells (3×10^5/well) were plated in triplicate and cultured *in vitro* with concanavalin A (1 μg/well) (Sigma, USA), Lb-Ag (10 μg/well), or in the absence of any stimulus as the negative control. The cell cultures were maintained for 72 h at 37 °C in a humidified atmosphere of 5% CO_2 in air. Then 16 h before harvesting, 1 Ci (3H)thymidine (Amersham International, UK) was added to each well, and the radioactivity uptake was measured in a scintillation counter (1600CA, Packard Instrument Company, Downers Grove, IL, USA). Results were expressed as stimulation indices (SI = average counts per minute [cpm] of stimulated triplicates with Lb-Ag/ average cpm of negative control) where values equal to or higher than 2·5 were considered as positive.

Macroscopic analysis of lymphoid organs

The spleens of 30 hamsters were excised and weighed in a precision balance. Spleens were visualized macroscopically and registered by digital photographs. Dissemination of parasites to spleens and other anatomic structures beyond the inoculation site was evaluated by macroscopic inspection (anatomical alterations) and confirmed by visualization of *Leishmania* through histopathological analysis of lymphoid organs. The draining popliteal lymph nodes of 10 animals and the spleens of 14 animals were subjected to microscopic evaluation.

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Histopathological analysis

Fragments from the skin ($n=24$) and the draining lymph nodes (popliteal, $n=10$) of the infected paw, as well as from spleen ($n=14$) and liver ($n=14$) were fixed in 10% buffered formalin and processed for paraffin embedding. Sections of 2–4 μm thickness were stained with haematoxylin-eosin and then observed by light microscopy (Nikon Eclipse E600 Microscope, Tokyo, Japan). The images were captured in CoolSNAP-Proc$_{CF}$ and edited by Image-Pro Plus program (Media Sybernetics, GA, USA). The results were expressed as a semi-quantitative analysis in which the main histopathological features were scored according to the number of animals in which the features were observed *vs* the number of animals analysed (given in parentheses) and scored according to the intensity of occurrence of the feature, varying from (−) absence; (±) slight presence of the histopathological feature; (+) moderate presence of the histopathological feature; to (++) full occurrence of the histopathological feature.

Statistical analysis

The data were analysed by Mann–Whitney test and Spearman's rank-correlation with the GraphPad Prism software version 4.00 for Windows (GraphPad Software, San Diego, CA, USA). The results were expressed as the mean ± standard deviation and median. Significant differences were considered when $P<0{\cdot}05$.

RESULTS

*Variances for cutaneous lesion increment post-*Leishmania braziliensis *infection*

All *L. braziliensis*-infected hamsters developed cutaneous lesions during the observational period (~ 110 days). The observation started at 2 weeks after infection, when characteristic inflammatory signs (erythema and oedema) were observed. After that there was a significant lesion increment. The first mean measure was 0·30 mm ± 0·30 mm (median = 0·22 mm, $n=34$); and the final mean measure was 2·12 mm ± 1·16 mm (median = 1·9 mm, $n=34$) ($P<0{\cdot}001$) (Fig. 1A). No spontaneous healing was observed in any animal.

A cutaneous ulcerated lesion was the most frequent clinical presentation (Fig. 1B and C). Elevated erythematous borders, granular aspect, with a necrotic surface (Fig. 1B), or recovered by crusts (Fig. 1C) were also commonly seen.

The variable pattern of lesion increment was quantified by a variance coefficient. We observed homogeneous (VC < 15%, $n=1$ experiment), intermediary (VC ⩾ 15% and VC ⩽ 30%, $n=2$ experiments) or heterogeneous (VC > 30%, $n=2$ experiments) patterns.

Histopathological findings of hamster cutaneous lesion

The skin histopathological findings ($n=24$) are summarized in Table 1. An intense inflammatory infiltrate reaching all over the dermis (Fig. 1D), consisting predominantly of macrophages in epithelioid arrangements, and large amounts of lymphocytes, characterizing a granulomatous reaction (Fig. 1E) was visualized in all animals. Neutrophils, plasma cells and eosinophils (sometimes inside macrophage vacuoles) were also seen (data not shown). Cytoplasmic vacuoles were observed in most macrophages (22/24), whereas *Leishmania* were seen in all of them (Fig. 1F). Areas of necrosis with calcification (11/24) (Fig. 1D) and Schaumann's bodies (lamellar basophilic structures) (22/24) (Fig. 1E and F) were seen. A positive association between lesion increment and the frequency of amastigotes was detected (Table 1).

Parasite dissemination to other anatomical compartments

No clinical evidence of cutaneous metastasis was observed. Draining popliteal lymph nodes ($n=10$ infected animals) were grey, with swollen and enlarged aspects in comparison to non-infected hamsters. They all presented disrupted architecture (10/10) with macrophages in epithelioid arrangement (Fig. 2A). Schaumann's bodies were often seen dispersed through the organ (9/10) (Fig. 2A). Macrophages exhibited vacuoles (10/10), and some of them showed moderate amounts of *Leishmania* (6/10), few amastigotes, or even degenerated parasites (4/10) (Fig. 2B).

Spleens were severely affected and some animals presented nodules ($n=8$) with a consistent aspect upon macroscopic visualization (Fig. 2C). The mean spleen weight was significantly higher ($P<0{\cdot}001$) in infected animals (0·47 g ± 0·32 g; median = 0·35 g; $n=20$) than in non-infected ones (0·13 g ± 0·04 g; median = 0·12 g; $n=10$). Indeed, a significant positive correlation between the spleen weight and lesion increment was observed in infected hamsters ($r=0{\cdot}62$; $P<0{\cdot}01$; $n=20$) (Fig. 2D). The spleen histopathology ($n=14$) showed mixed inflammatory infiltrate in all animals analysed, white pulp rarefaction (13/14), presence of granuloma (11/14) (Fig. 2E), vacuolated macrophages (11/14) and presence of *Leishmania* amastigotes (6/14) (Fig. 2F).

Although the macroscopic aspect of the liver seemed normal, we observed a number of histological abnormalities. The main findings were the presence of mixed inflammatory infiltrate (10/14), composed predominantly of mononuclear cells, mainly around the perivascular area (Fig. 3A). In these cases *Leishmania* parasites were not seen but vacuolated macrophages with acidophilic contents suggestive of amastigote forms were detected (5/14) (Fig. 3A).

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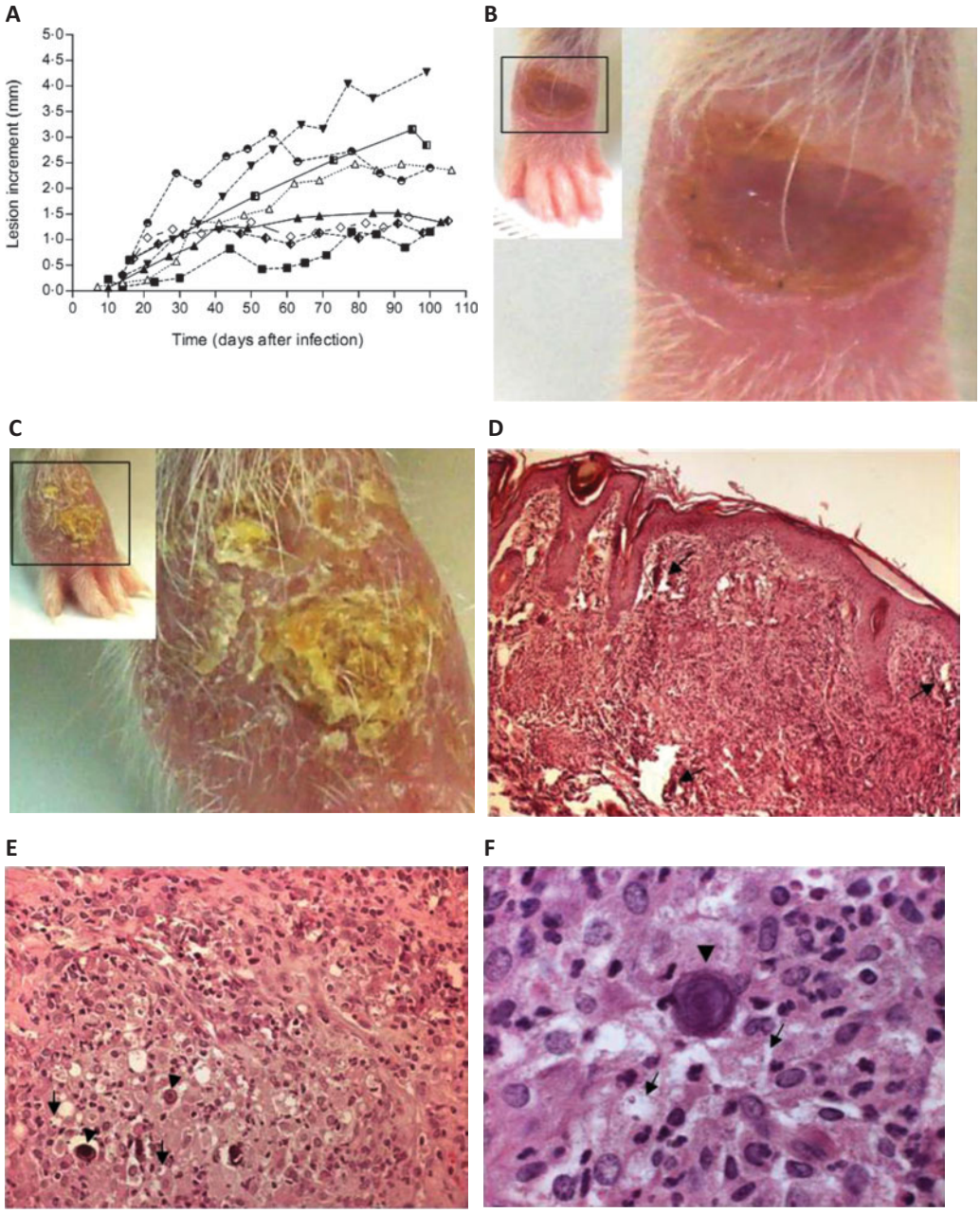

Fig. 1. Evaluation of the clinical course, macroscopic and histopathological aspects of skin lesions from golden hamsters infected with *Leishmania* (*Viannia*) *braziliensis*. (A) Lesion increment development in each of 8 independent experiments. Each line represents 1 experiment and the points indicate the average of lesion increments in each group. (B and C) Macroscopic aspect of the infected paw at approximately 110 days after *Leishmania* infection. Slides of representative lesions localized at the site of parasite inoculation (dorsal face of back right paw) taken at the end of the observational period (110 days). (D) Mixed inflammatory infiltrate reaching all over the dermis; pointed arrows indicate areas with calcification necrosis (skin, 20×). (E) Macrophages in epithelioid arrangement; arrowheads indicate Schaumann's bodies; pointed arrows indicate vacuolated macrophages with *Leishmania* inside (skin, 40×). (F) Granuloma; pointed arrows indicate cytoplasmatic vacuoles containing amastigotes inside; arrowheads indicate Schaumann's bodies (skin, 100×).

Blood lymphocyte proliferation response (LPR) to leishmanial antigens may relate to the skin lesion severity

A total of 21 out of 22 infected hamsters had a positive LPR. The stimulation index (SI) to Lb-Ag was quite variable and ranged from 4·2 to 137 (mean = 33·8 ± 42·3; median = 15·9; $n = 22$) (Fig. 3B). SI to Lb-Ag were negatively associated with lesion increments of infected animals at the end of the monitoring period ($P < 0{\cdot}05$; $r = -0{\cdot}50$) (Fig. 3C). The mean SI to mitogen was similar when infected (147 ± 120; median = 100; $n = 22$) and non-infected hamsters (198 ± 168; median = 199; $n = 9$) were compared (Fig. 3B).

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Table 1. Histopathological features of spleen and skin lesions from *Leishmania* (*Viannia*) *braziliensis* infected golden hamsters

Histopathological aspects	Tissues	Independent experiments				
		Exp 1 ($n=5$)	Exp 2 ($n=5$)	Exp 3 ($n=4$)	Exp 4 (n=5)	Exp 5 ($n=5$)
Mixed inflammatory infiltrates	Skin	++ (5/5)[a]	++ (5/5)	++ (4/4)	++ (5/5)	++ (5/5)
	Spleen	na ()	na ()	++ (4/4)	+ (5/5)	+ (5/5)
Granulomatous reactions	Skin	++ (5/5)	++ (5/5)	++ (4/4)	++ (5/5)	++ (5/5)
	Spleen	na ()	na ()	++ (4/4)	+ (3/5)	+ (4/5)
Vacuolated macrophages	Skin	+ (5/5)	+ (5/5)	+ (4/4)	± (4/5)	+ (4/5)
	Spleen	na ()	na ()	++ (4/4)	+ (4/5)	+ (3/5)
Leishmania amastigotes	Skin	+ (5/5)	± (5/5)	++ (4/4)	± (5/5)	± (5/5)
	Spleen	na ()	na ()	± (3/4)	± (2/5)	± (1/5)
Schaumann's bodies	Skin	+ (3/5)	± (5/5)	± (3/4)	+ (3/5)	+ (3/5)
	Spleen	na ()	na ()	± (1/4)	− (5/5)	− (5/5)
Necrosis with calcification	Skin	+ (2/5)	− (5/5)	± (1/4)	± (2/5)	± (1/5)
	Spleen	na ()	na ()	− (4/4)	− (5/5)	− (5/5)
Tissue disarrangement architecture	Skin	++ (5/5)	++ (5/5)	++ (4/4)	++ (5/5)	++ (5/5)
	Spleen	na ()	na ()	++ (3/4)	± (4/5)	+ (4/5)
Clusters of macrophages	Skin	++ (5/5)	++ (5/5)	++ (4/4)	++ (5/5)	++ (5/5)
	Spleen	na ()	na ()	++ (3/4)	± (3/5)	+ (4/5)
White pulp rarefaction	Spleen	na ()	na ()	++ (4/4)	+ (5/5)	+ (4/5)
Paw lesion increment at final measure (mm)		2·30	1·34	4·27	1·36	1·44

n, Number of animals per group.
[a] Number of animals in which the histopathological feature was observed / number of animals analysed.
(−)Absence; (±) slight presence of the histopathological feature; (+) moderate presence of the histopathological feature; (++) full occurrence of the histopathological feature.
na, Not analysed.

*Levels of anti-*Leishmania *IgG correlated with infection severity*

Anti-*Leishmania* IgG was detected in all infected animals. As expected, the OD values were significantly higher in infected (3·41 ± 0·22; median = 3·47; $n=29$) than non-infected animals (0·58 ± 0·27; median = 0·5; $n=13$; $P<0·001$). The anti-*Leishmania* IgG levels positively correlated with lesion increments in the hamsters' paws (r = 0·45; $P<0·05$; $n=25$) (Fig. 3D) and with spleen weight (r = 0·66; $P<0·01$; $n=20$) (Fig. 3E).

DISCUSSION

The fact that the golden hamster presents susceptibility to dermotropic *Leishmania* (*Viannia*) species makes this species a better experimental model for ATL studies than the murine model, as the latter is naturally resistant to these strains (Rey *et al*. 1990; De Oliveira *et al*. 2004). However, different *Leishmania* isolates or species and/or protocols have been used that do not allow prediction of the outcome of infection in this animal model as opposed to other well-established mouse/*Leishmania* models. Herein, all infected animals were susceptible to *L. braziliensis* and developed skin-ulcerated lesions. The severity of the infection in this model correlated to spleen weight, the intensity of lymphocyte proliferation to leishmanial antigens, and the titres of anti-*Leishmania* antibodies.

The *Leishmania* infection protocol used in this study was able to generate cutaneous lesions in all animals. Thus, it could be recommended for clinical, vaccine or therapeutic studies. The inflammatory signs appeared early in the course of the disease and were already observed within the first 3 weeks after infection. At 60 days post-infection all animals presented signs of disease. Shorter or longer prepatent periods were observed by others (Wilson *et al*. 1979; Kahl *et al*. 1990, 1991). In our study, ulcerated lesion was the main macroscopic clinical presentation after 4 months of infection. This aspect, together with the chronic state of the disease, closely resembles non-healing human CL and possibly reproduces some of the immunopathological aspects of the human disease.

Although all animals developed skin lesions, the final measures of infected paws differed among intra- or inter-independent experiments. The variability coefficient analysis tended to show an intermediary variance when animals under the same experimental infection conditions were compared. This result is expected to occur in hamsters because of the outbred genetic background. However, variability is also observed even when isogenic mice are used in experimental leishmaniasis (Pereira *et al*. 2009). Therefore, variability in lesion increment has to be taken into

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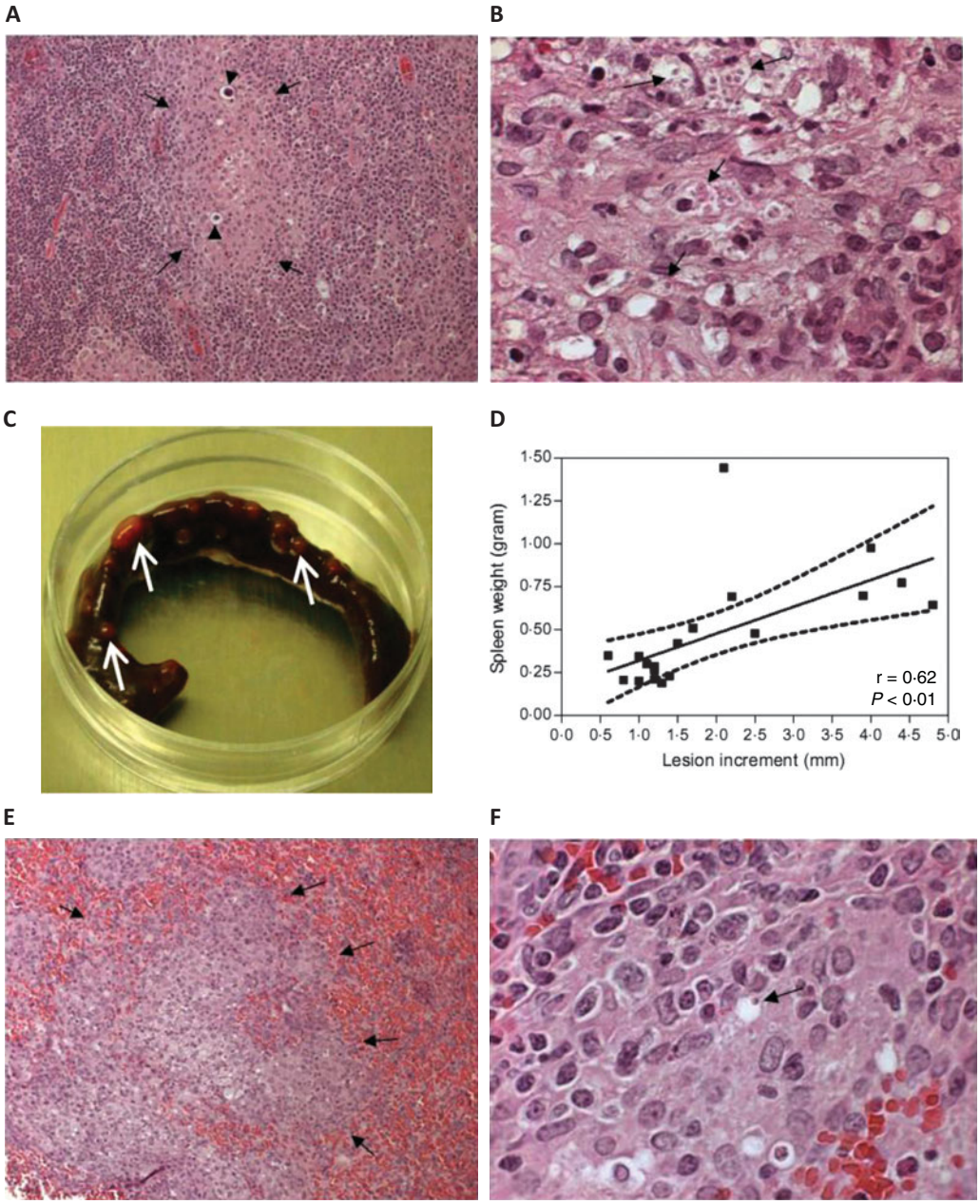

Fig. 2. Macroscopic and histopathological aspects of secondary lymphoid organs from golden hamster infected by *Leishmania* (*Viannia*) *braziliensis*. (A) Pointed arrows indicate macrophages in epithelioid arrangement; arrowheads indicate Schaumann's bodies (lymph node, 20×). (B) Granulomatous reaction; pointed arrows indicate vacuolated macrophages containing amastigotes (lymph node, 100×). (C) Slide of representative picture of spleen from infected animal. White arrows point to some of the several nodules found in infected spleen. (D) Correlation between spleen weight and lesion increment in paws from 20 infected animals after approximately 110 days of infection. The correlation graph shows fit line with confidence curve. r = correlation coefficient; P = significance level. (E) Pointed arrows indicate epithelioid macrophages characterizing granulomatous reaction (spleen, 20×). (F) Pointed arrows indicate *Leishmania* inside vacuolated macrophage in granuloma (spleen, 100×).

consideration when lesion size is a parameter to evaluate drug response or vaccine protection.

Besides the skin macroscopic aspects, another striking finding is the similarity of the skin histopathological changes when *L. braziliensis*-infected hamsters were compared (Laurenti *et al*. 1990; Kahl *et al*. 1991; Sinagra *et al*. 1997) with human lesions (Magalhães *et al*. 1986). As observed in CL lesions, granulomatous reactions consisting predominantly of epithelioid macrophages, lymphocytes and moderate amounts of plasma cells and scarce amounts of amastigotes were consistently seen in our experimental model. On the other hand, moderately parasitized macrophages were detected in severely clinically compromised animals. A limitation of this study was not to include the parasitic load results. The high bacterial contamination levels (probably from lesion origin) in the axenic cultures did not enable

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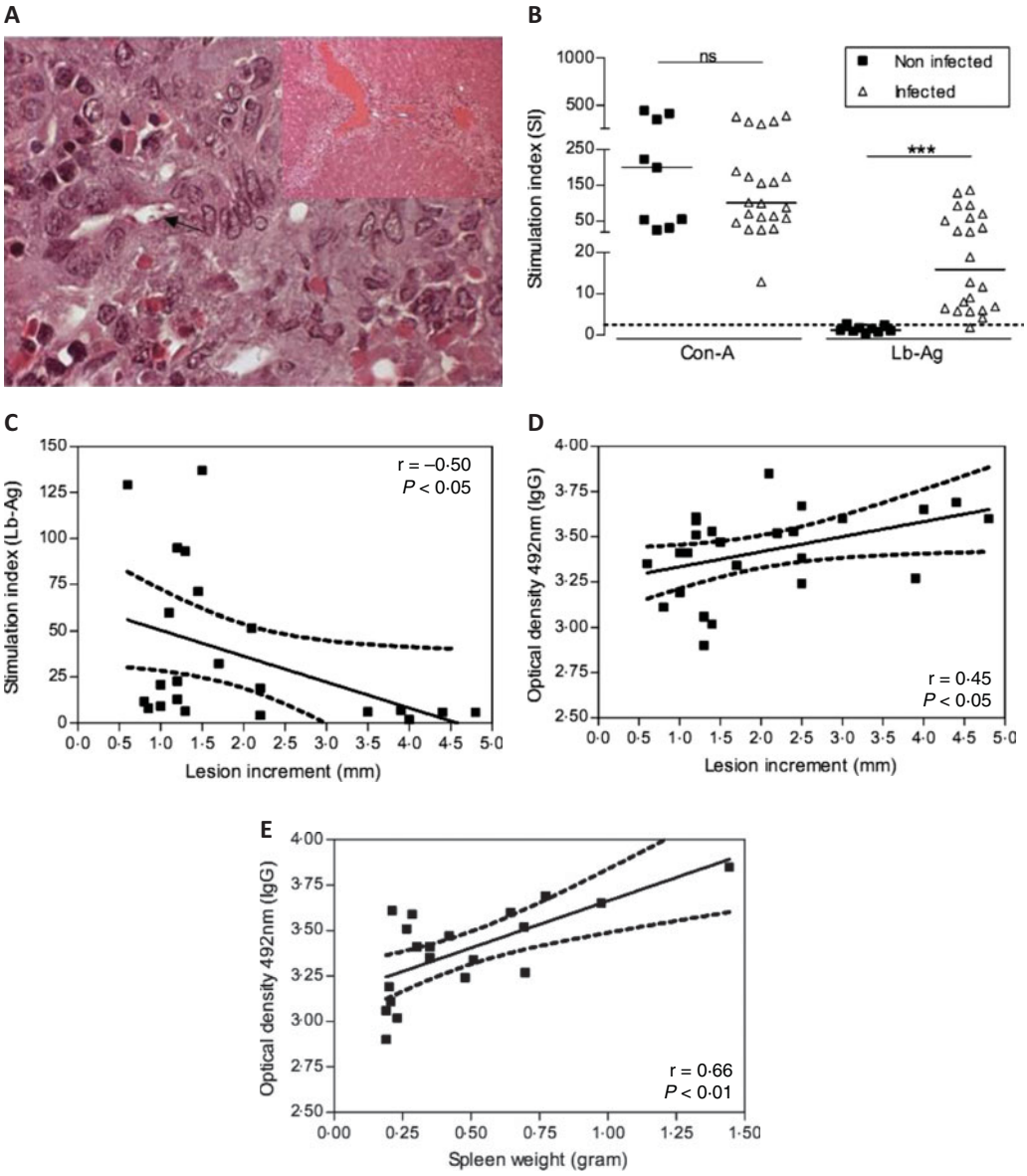

Fig. 3. Histopathological aspects of liver and immunological responses to leishmanial antigens of golden hamster infected with *Leishmania* (*Viannia*) *braziliensis*. (A) Mixed inflammatory infiltrate around perivascular area (window, liver, 20×); macrophages in epithelioid arrangement; pointed arrows indicate forms of amastigotes inside vacuolated macrophage (liver, 100×). (B) Lymphocyte proliferative assays using peripheral blood mononuclear cells (PBMC) obtained from uninfected and infected animals. Cells were stimulated *in vitro* with *L. braziliensis* antigens (Lb-Ag) or mitogen concanavalin A (Con-A). The results were expressed as a stimulation index (SI). *** $P<0{\cdot}001$. ns, not statistically significant. Each point represents 1 animal and the horizontal bar indicates the median. Dotted line indicates the cut-off SI = 2·5. (C) Correlation between lesion increment and lymphocyte proliferation intensity (stimulation index) under *Leishmania* antigen (Lb-Ag) stimuli. (D) Association between lesion increment in hamster paws and anti-*Leishmania* IgG levels (optical density values). (E) Correlation between spleen weight (in grams) and the anti-*Leishmania* IgG levels (optical density values). The correlation graphs (C, D and E) show fit lines with confidence curves. r = correlation coefficient; P = significance level.

calculation of the frequency of parasites in skin-infected tissues by limited dilution assay. In the future, molecular assays such as real-time PCR to quantify *Leishmania* DNA products can be used.

Schaumann's bodies were a frequent finding especially in skin and lymph nodes, as observed by other authors (Kahl *et al*. 1990, 1991; Laurenti *et al*. 1990). This structure has been associated with a deficient phagocytic macrophage system (Laurenti *et al*. 1990). Curiously, although Schaumann's bodies are commonly seen in infected hamsters, they are not usually described in humans or in other experimental animals with leishmaniasis (Essayag *et al*. 2002). In any case, the presence of Schaumann's bodies even in the absence of parasites strongly suggests *Leishmania* infection (Ribeiro-Romão *et al*. unpublished data).

In our experimental protocol, a greater cutaneous lesion increment was accompanied by systemic

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clinical abnormalities observed in the spleen, lymph nodes, and liver involvement (data not shown). *Leishmania* visceralization has been reported in hamsters infected by *Viannia* and *Leishmania* dermotropic *Leishmania* species (Rey *et al*. 1990; Almeida *et al*. 1996; Sinagra *et al*. 1997; Soliman, 2006) especially when infective inoculum is high. Although skin metastases were detected in *L. braziliensis* infected hamters (Brazil, 1976; Wilson and Lollini, 1980), the parasite isolate used herein did not induce metastases, agreeing with previous reports (Travi *et al*. 1988).

The LPR assay has become an alternative for immunological studies in hamster models because of the low availability of anti-hamster monoclonal antibodies against cytokines or surface molecules. Although spleen and lymph node compartments have been frequently used as the source of mononuclear cells for LPR assays (Osorio *et al*. 2003; Dea-Ayuela *et al*. 2007), we have chosen blood cells. Our rationale was to rule out the possibility that *Leishmania* antigens coming from these lymphoid organs may cause *in vitro* cell stimulation, prejudicing stimulation index calculation. As far as we know, blood cells for analysing the immune response to leishmanial antigens have not been previously used in the hamster model.

The intensity of LPR under leishmanial stimuli inversely correlated with disease severity. Similarly, snout infection by *L. panamensis* is accompanied by a low lymphoproliferation intensity (Osorio *et al*. 2003). However, animals with small lesion increments also presented low LPR results whereas CL patients presenting low LPR stimulation indices are at high risk for cutaneous lesions relapses (Mendonça *et al*. 1986). This suggests that any deficiency in the effector cellular immune response could impair the parasite clearance.

The detection of anti-*Leishmania* antibodies has been used as a promising biomarker to assess the clinical course of leishmaniasis (Gomes-Silva *et al*. 2009). Here, we showed an association between IgG anti-*Leishmania* levels and disease severity, similar to the results previously shown in *L. panamensis* infection (Osorio *et al*. 2003). In our study, the anti-*Leishmania* antibody titres directly correlated with lesion increment and also with spleen weight.

Recently a molecular assay based on quantification of RNA transcripts has been used for the detection of cytokines, chemokines and cell-surface markers in hamster models (Espitia *et al*. 2010) as an important strategy for evaluating the immune response. This model may also allow associations between clinical outcome and more reliable immunological findings.

Here we showed that the clinical and immunological reactions can vary among independent experiments even when a single isolate of *L. braziliensis* is used to infect golden hamsters. Also, skin lesion increments, splenomegaly and the proliferative capacity of lymphocytes reactive to *Leishmania* and IgG anti-*Leishmania* levels correlated with disease severity. Our results indicate that the golden hamster is an appropriate model for immunopathogenesis studies, and support its use in clinical, vaccine and chemotherapy experimental protocols of cutaneous leishmaniasis caused by *L. braziliensis*.

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